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EXPERIMENTAL STUDY OF THYROTOXIC SERUM.*

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INTRODUCTION.

It is well known that complete removal of the thyroid gland is followed by a typical group of symptoms, terminating in death. More recently it has been shown by a number of observers that the acute symptoms, simulating tetany, and the rapid fatal termination follow the removal of the parathyroid bodies, even though the entire thyroid is left *in situ*. On the other hand, the removal of the thyroid, leaving the parathyroids untouched, is followed by chronic symptoms, such as myxedema, cretinism, and cachexia.

The present work was begun in the hope that it might be possible to produce similar clinical pictures by means of specific cytotoxins, selective in their action on the thyroid and parathyroid cells.

Preliminary reports of Mankovsky¹ and Gontscharukov² lent encouragement to this view. Both stated that they had produced thyrotoxic sera, injections of which produced symptoms simulating tetany, the thyroid cells showing microscopic evidences of degeneration. Mankovsky introduced the thyroids of dogs into the peritoneal cavity of cats, at intervals of two weeks, and after the third injection bled the cats and separated their serum, which proved toxic to dogs.

* Received for publication October 15, 1903.

¹ *Russ. Arch. f. Path.*, 1902, 14, 571-591.

² *Centralbl. f. allg. Path. u. path. Anat.*, 1902, 13, 121-124.

Gontscharukov also used the thyroids of dogs, injecting them into rams. He, too, produced a serum which he concluded was thyrotoxic. Neither of these workers, so far as I can learn, has published a subsequent full report, and as their experiments were conducted on a few dogs only, their work cannot be regarded as conclusive.³

While many problems have arisen during the progress of my experiments that demand much more study than I have been able to give them, the results so far obtained appear of enough importance to merit publication at this time.

METHODS OF IMMUNIZATION.

Goats and dogs were used for my experiments. At first an emulsion of goats' thyroids was prepared and injected into the peritoneal cavity of goats. The thyroids were removed, with all aseptic precautions, cut up into small pieces, and placed in a mortar containing some purified sand. The sand was prepared by digesting with weak hydrochloric acid for twenty-four hours, to remove carbonates and render other salts soluble, then washed repeatedly with distilled water until all traces of the acid were gone, and finally incinerated to destroy all organic matter.

On account of the tough, fibrous nature of the thyroid, it was found necessary to continue the grinding for several hours; to prevent contamination, the mortar was covered with a sheet of asbestos, perforated to allow the passage of the pestle. When the mass was completely pulpified, a small amount of sterile salt solution (0.85 per cent.) was added and the mixture stirred. The supernatant portion was pipetted off to get rid of the sand, and then strained through a fine wire gauze to remove the larger pieces of tissue. One obtains in this manner an emulsion containing not only a preponderance of thyroid cells, but also a small amount of various cells, common to all tissues.

Increasing doses of emulsion were injected into the peritoneal cavity of goats, at intervals of ten to fourteen days; at first one dog's thyroid was used, then two, and so on, until finally ten thyroids were used for a single injection. After the third injection, the study of the sera of the immunized animals was begun. The immunization was kept up, never allowing an interval of more than one month to elapse between injections. Aside from a slow progressive loss in weight, no effect was noticed on the goats.

Later in the work it was thought best to remove all of the blood from the thyroids before making an emulsion, and this was readily accomplished by washing out the gland *in situ*, through the thyroid artery, using a sterile

³The report by J. L. YATES (*Univ. of Penna. Med. Bull.*, 1903, 16, 195-200) on the experimental production of cytolsins for the adrenal, thyroid, and parathyroid glands of dogs appeared after the present report had been placed in the hands of the editors. Also the report of W. G. MACCALLUM (*Med. News.*, Oct. 31, 1903), "On the Production of Specific Cytolytic Sera for Thyroid and Parathyroid."

normal salt solution. The washing was continued until the gland became completely bloodless. That this was accomplished was apparent also in microscopic sections of washed thyroids. One of the goats was immunized with injections of washed thyroids.

For purposes of comparison, one goat was immunized with an extract of dog's thyroid, known as "colloid matter." This was prepared according to Hutchinson's method, by digesting sterile crushed thyroids with sterile magnesium sulphate solution (5 per cent.) for six hours. Then the mixture was thoroughly centrifugalized to throw down all undissolved matter, and the clear supernatant liquid pipetted off. To this fluid a few drops of acetic acid were added, and a heavy whitish flocculent precipitate was thrown down, which was separated and washed to remove all of the acetic acid. A suspension of this precipitate — the colloid matter — was made in normal salt solution, and injected into the peritoneal cavity of a goat at intervals of ten days, using an increasing number of thyroids, as in the previous experiments.

Method of bleeding.—As considerable difficulty was met with in bleeding the goats, a special Erlenmeyer flask, with a small glass tube blown into its side near the neck, and another at the bottom, was used. To the arm at the bottom an inspiring needle is fastened, and capped with a test-tube. The upper arm is plugged with cotton, and serves as the place of attachment for the aspirating syringe. The flask is fitted with a rubber cork, and to keep the clot from sliding about two glass rods are inserted. The blood is easily withdrawn from the superficial jugular vein, which stands out prominently, with a little pressure at the side of the neck, near the anterior border of the sterno-cleido-mastoid muscle. The goats stand the bleeding very well, and as much as 400 c.c. were withdrawn at one time. The blood clots very quickly, and the serum is easily removed by slipping the needle off and pouring the serum into sterile flasks.

EXPERIMENTS WITH THE SERUM OF GOATS IMMUNIZED WITH DOGS' THYROIDS.

For control purposes, several dogs were given intravenous injections of normal goat serum, and as much as 7 c.c. per kilogram weight was found without any effect. The animals were not at all depressed, showed no abnormal symptoms, and when killed one or two weeks later there were no microscopic changes anywhere.

The dogs injected with the immune sera may be divided into three groups. Group I (see Table I) received injections of serum from goats immunized with thyroid tissue; Group II (see Table II) received injections of the same serum as the former, but it was first treated with dogs' red blood corpuscles to remove the hemolytic amboceptors; and finally Group III (see Table III) consisting of dogs injected with serum from the goat immunized with "colloid matter."

TABLE I.

Dogs Injected with Serum of Goats Treated with Dog's Thyroid.

Dog No.	Weight	Dose of Toxic Serum per Kilogram	Reinjected	Results
1	9 kg.	Jan. 22, 3.5 c.c. per kg., intravenous.		Nausea, vomiting, defecation, urination, and marked depression; <i>hemoglobinuria</i> . Lived ten hours.
3	5 kg.	Feb. 3, 2 c.c. per kg., intravenous.	Feb. 10, 3 c.c. per kg., intravenous.	After first injection, depression for several hours, but rallied. The next day very stupid, watering of eyes; <i>hemoglobinuria</i> ; slowly improved. After second injection, dog went into convulsions and died within one hour. Lived seven days after first injection.
4	7.5 kg.	Feb. 3, 2 c.c. per kg., intravenous.		Vomiting, frequent defecation, depression, watering of eyes, and stupid for several days; <i>hemoglobinuria</i> . Killed on sixth day.
5	3.5 kg.	Feb. 10, 3 c.c. per kg., intraperitoneal.		Shortly after injection, clonic convulsions which lasted several minutes; then animal walked about with marked spastic gait in hind legs. Next day, watering of eyes, frequent general tremors; quite sick; <i>hemoglobinuria</i> . Lived two days.
7	8 kg.	March 11, 3 c.c. per kg., intravenous.	March 17, 6 c.c. per kg., intravenous.	Depressed for a few days after first injection; watering of eyes; <i>hemoglobinuria</i> . After second injection, convulsions and death in two hours. Lived six days after first injection.
8	5.5 kg.	April 4, 4 c.c. per kg., intravenous.	April 5, 4 c.c. per kg.; April 11, 5 c.c. per kg.	Not very sick after first injection, but stupid and depressed for several days after second injection. After third, convulsions and death in one hour; <i>hemoglobinuria</i> . Lived seven days after first injection.
9	8 kg.	April 24, 4 c.c. per kg., intravenous.		Vomiting, frequent defecation; depressed and stupid; <i>hemoglobinuria</i> . Lived six hours.
10	7.5 kg.	April 29, 2 c.c. per kg., intravenous.		Not very sick; watering of eyes; slight <i>hemoglobinuria</i> . Killed on fifth day.
11	8 kg.	May 13, 1 c.c. per kg., intravenous.		Vomiting, and slight convulsions; then marked depression and death in eight hours. Lived eight hours.
13	6 kg.	May 20, 3 c.c. per kg., intravenous.	May 21, 5 c.c. per kg., intravenous.	Vomiting, frequent defecation, marked spastic gait in hind legs; watering of eyes; <i>hemoglobinuria</i> . After second injection, convulsions and death in one hour. Lived twenty-four hours after first injection.
15	9 kg.	May 25, 4 c.c. per kg., intravenous.	June 2, 4 c.c. per kg., intravenous.	Depressed and stupid for several days; watering of eyes; <i>hemoglobinuria</i> ; feces dark color. After second injection, convulsions and death in two hours. Marked loss in weight. Lived eight days after first injection.
17	9 kg.	June 3, 4 c.c. per kg., intravenous.	June 10, 5 c.c. per kg., intravenous.	Stupid and sick for several days; watering of eyes; <i>hemoglobinuria</i> ; feces dark red; then improved; but after second injection grew rapidly weak and cachectic, and died June 23. Lived twenty days after first injection.

TABLE I—*Continued.*

Dog No.	Weight	Dose of Toxic Serum per Kilogram	Reinjected	Results
19	5 kg.	June 24, 0.5 c.c. per kg., intravenous.	June 30, 1 c.c. per kg., intravenous.	Not much affected by first injection. After second, convulsions and death in thirty minutes; <i>hemoglobinuria</i> . Lived six days after first injection.
20	5.5 kg.	June 24, 4 c.c. per kg., intravenous.		Vomiting, frequent defecation, convulsions, and death in one hour. Lived one hour.
21	8 kg.	June 24, 2 c.c. per kg., intravenous.	June 30, 1 c.c. per kg., intravenous.	Depressed and stupid, and remained so; lost weight constantly, and became quite weak; <i>hemoglobinuria</i> ; watering of eyes. Died July 14. Lived twenty days after first injection.
23	7.5 kg.	June 30, 1.5 c.c. per kg., intravenous.		Vomiting, convulsions; death in thirty minutes.
24	8 kg.	June 30, 1 c.c. per kg., intravenous.	July 11, 1 c.c. per kg., intravenous.	Vomiting, diarrhea, depression; remained stupid for several days; watering of eyes; <i>hemoglobinuria</i> . After second injection, convulsions and death in one hour. Lived eleven days after first injection.
29	5 kg.	July 13, 7 c.c. per kg., intraperitoneal.		Vomiting, frequent defecation, depression. Found dead next morning—numerous subperitoneal ecchymoses, and some blood in peritoneal cavity; <i>hemoglobinuria</i> .
31	11 kg.	July 27, 7 c.c. per kg., intraperitoneal.		Depressed, vomiting, watering of eyes; <i>hemoglobinuria</i> . Improved and became quite lively. Killed on Aug. 4. Lived eight days.

1. *Clinical picture.*—Group I: These dogs received subcutaneous, intraperitoneal, and intravenous injections of immune serum, in varying amounts, averaging about 4 c.c. per kilogram. Although injections under the skin and into the peritoneum gave toxic results, still they were usually complicated by exudation and hemorrhage, and hence the intravenous method was most frequently used. During the injection the dogs became depressed, and shortly after began to vomit repeatedly; they emptied their bowels and bladder, lay about stupidly, or walked with a marked spastic gait in the hind legs. Then they either became rapidly worse, developing convulsions and coma, and dying in one-half to two hours, or recuperated somewhat, remaining sick, stupid, and listless for a few days, and then slowly improving. All the dogs had a marked hemoglobinuria, which appeared in about six hours and continued for several days. The feces were frequently dark and blood-stained. Shortly after the injection lachrymation

was noticed, and this continued for several days; frequently a conjunctivitis developed. A few hours after the injection a slight rise in temperature of one or two degrees was frequent. On the second or third day, at times some stiffness of the hind legs was noticed in walking, and Trousseau's phenomenon was easily produced. But it is not at all uncommon for normal dogs to show a similar spasm of the hind legs when irritated by pressure. No spontaneous convulsions, such as Mankovsky and Gontscharukov described on the third or fourth day, was noticed in these dogs. Some of the dogs lived four or five weeks, and during this time lost markedly in weight, and became cachectic and weak.

TABLE II.
Dogs Injected with Immune Goat Serum Previously Treated with
Dog's Corpuscles.

Dog No.	Weight	Dose per Kilogram of Toxic Serum Treated with Blood Corpuscles of Dog	Reinjected	Results
6	9 kg.	Feb. 12, 1.8 c.c. per kg., intraperitoneal.	Feb. 19, 2.5 c.c. per kg., intraperitoneal.	Not much affected by first injection, but depressed and stupid for several days after second; <i>trace of hemoglobinuria</i> ; watering of eyes; loss in weight. Killed on thirteenth day.
12	10 kg.	May 14, 3.5 c.c. per kg., intravenous.		Vomiting, frequent defecations and convulsions, and died in one hour.
16	7.5 kg.	May 26, 4 c.c. per kg., intravenous.	June 22, 4 c.c. per kg., intravenous.	Not much affected by first injection; marked depression after second, but rallied slightly, and then in a few hours convulsions and death in eight hours. Watering of eyes; <i>trace of hemoglobinuria</i> . Lived seven days after first injection.
18	4 kg.	June 11, 2 c.c. per kg., intravenous. July 14, 2.5 c.c. per kg.	July 1, 1.5 c.c. per kg., intravenous. July 14, 2.5 c.c. per kg.	Slightly depressed after first injection and remained stupid and listless for several days; watering of eyes; <i>slight hemoglobinuria</i> ; loss in weight; gait staggering. After second injection, previous symptoms exaggerated; cachexia developed. After third injection, marked depression, and found dead next morning. Lived thirty-three days after first injection.
22	8 kg.	June 24, 2 c.c. per kg., intravenous.	July 1, 4 c.c. per kg., intravenous.	Vomiting and slight depression after first injection, watering of eyes, listless; <i>trace of hemoglobinuria</i> . After second injection, convulsions, and death in one hour. Lived seven days after first injection.
30	7.5 kg.	July 14, 3 c.c. per kg., intravenous.		Slight depression, watering of eyes, listless; <i>slight hemoglobinuria</i> ; constant loss in weight. Killed Aug. 20. Lived thirty-seven days.

Group II: These dogs received intraperitoneal and intravenous injections of immune serum, which had previously been digested with washed dog's corpuscles at 0° C. for thirty minutes. The dogs were not quite as sick as those in the previous group, but, although milder, the clinical picture resembled closely that of the former. A slight hemoglobinuria was produced.

TABLE III.

Dogs Injected with the Serum of Goats Treated with Colloid Matter.

Dog No.	Weight	Dose per Kilogram Colloid Matter Toxic Serum	Reinjected	Results
26	6.5 kg.	July 7, 6 c.c. per kg., intravenous.		Convulsions, and death in thirty minutes.
28	6 kg.	July 8, 2 c.c. per kg., intravenous.	July 13, 3 c.c. per kg., intravenous. Aug. 4, 5 c.c. per kg.	Vomiting, diarrhea, trembles all over, stupid and listless; recovered and became lively. Not much affected by subsequent injections; lost constantly in weight. Killed Aug. 15. Lived thirty-eight days after first injection.
32	7 kg.	July 28, 5 c.c. per kg., intravenous.	Aug. 4, 8 c.c. per kg., intraperitoneal.	Not very much affected; nausea; next day listless and stupid; some rigidity in hind legs; watering of eyes. Found dead next morning after second injection. Lived seven days after first injection.
33	8 kg.	July 29, 3 c.c. per kg., intravenous.	Aug. 8, 3 c.c. per kg., intravenous.	Depressed, listless for several days; collapse after second injection, but recovered. Killed Aug. 21. Lived twenty-three days after first injection.
34	9 kg.	Aug. 13, 4 c.c. per kg.		Not much affected; quite lively next day. Killed Aug. 21. Lived eight days after first injection.
35	7 kg.	Aug. 13, 7 c.c. per kg.		Vomiting, defecation, stupid and listless; but slowly improved. Killed Aug. 21. Lived eight days after first injection.

Group III: These dogs were injected intravenously with the serum of the goat, immunized with "colloid matter." A similar picture, but milder, was also observed in these animals. There was not, however, any demonstrable hemoglobinuria.

2. *Anatomical changes.*—Macroscopic: The post-mortem appearances varied with the mode of injection. The dogs receiving toxic serum subcutaneously showed in early cases a marked localized edema, and in those that survived several days a cyst-like accumulation of a bloody serous fluid was found. When the injections were given intraperitoneally, at first multiple subperitoneal

hemorrhages were found, but if the dog survived several days, a plastic peritonitis developed, in the exudate of which the colon bacillus was present. The conclusion naturally was drawn that the serum was endotheliotoxic, and permitted the colon bacillus to invade the peritoneum. The majority of the dogs received intravenous injections, and a rather constant picture presented itself after death. There was always a marked general pallor; flabby heartmuscle; enlarged, bloody spleen; swollen, pale, fatty kidneys; and in the early stages the liver was swollen and chocolate-colored, in the latter yellow and fatty. The thyroid was frequently flabby, but otherwise it showed no changes.

Microscopic: Frozen sections, prepared from the various organs and stained with sudan III, showed fatty changes frequently in the thyroid and heart, and constantly in the liver and kidneys. Teased preparations revealed cloudy swellings in the same organs.

Thyroid gland, parathyroids, and hypophysis: The thyroid glands of normal and injected dogs were hardened in Flemming's fluid and were studied unstained for the fat-reacting granules described by Erdheim.⁴ The granules were found to be present and similar in all the glands.

The thyroids of the dogs injected with toxic serum show a decrease in colloid, which frequently is entirely absent and seems to have disappeared rapidly. The capillaries are often enlarged, and hemorrhages into the alveoli are occasionally observed. The epithelium presents a variable picture. Often the nuclei are swollen and vacuolated. In more marked cases, a partial or complete destruction of some of the cells and a marked desquamation of the remaining ones have occurred, so that the alveolus is distended with the dying cells and their fragments (see Plate IV, Figs. 1 and 2). In a number of the dogs dying later the epithelium has proliferated and filled the lumen with branched papillary-like processes, quite similar to those described by Halsted⁵ in his cases of compensatory hypertrophy following partial thyroidectomy (Plate V, Figs. 1 and 2). In no cases were there any recognizable changes in the parathyroid bodies. The same is

⁴ *Beitr. zur path. Anat. u. z. allg. Path.*, 1903, 33, 158-236.

⁵ *Johns Hopkins Hosp. Rep.*, 1896, 1, 373.

also true of the hypophysis which was studied most carefully in serial sections by Dr. Le Count.

Liver: This shows marked active congestion in the dogs that succumbed in a short time. In those that survived a day or more the liver cells show various stages of degeneration, and several of the specimens present scattered areas of insular necrosis in which all semblance to cell structure is lost, the areas consisting of débris with skeletons of red blood corpuscles. Frequently large amounts of pigment are visible. Marked fatty changes are frequent.

Kidney: The kidneys show distinct changes. In animals that have lived a short time there is marked hyperemia, with occasional glomerular hemorrhages. The epithelium in those that survived a day or more is loaded with granules of various sizes and of a deep-brown color. In places these granules resemble fragments of red blood corpuscles. Where the cells appear more normal the lumen is filled with a dark-brown homogeneous mass, evidently a fusion of granules discharged from the living cells. In a fortunate section this change can be traced in a single tubule. The glomerulus is frequently crowded to one side and the capsule distended by a finely granular material. Acute degenerative changes, desquamation of the epithelial cells, and pronounced fatty changes are not uncommon.

Spleen, lymph glands, bone marrow: The sections of the spleen are usually loaded with red blood corpuscles in various stages of degeneration, and blood pigment is abundant. Numerous accumulations of red blood corpuscles, within a retaining membrane, are seen. The corpuscles are often fused into a brownish mass similar to that described in the kidney tubules, and others appear merely as shadows. Whether all these masses are phagocytic cells is hard to say definitely, as some appear as if composed of agglutinated red blood cells.

No marked changes were observed either in the lymph glands or bone marrow. The latter has not been studied as thoroughly as it ought to have been. In some of the glands are large phagocytic cells filled with disintegrating red corpuscles.

Pancreas, and adrenals: In neither of these were there any marked changes.

Nervous system: Dr. Rothstein examined the cords and brains of several dogs, with wholly negative results.

The changes in the dogs injected with "colloid matter immune serum" are not as marked as those described, but still they are similar. The most striking and constant feature is the marked or complete loss of colloid matter in the acini of the thyroid gland.

3. *Studies of the action of the immunized goat serum in vitro.*—Cytolysis and agglutination of thyroid cells: The technic described by Flexner and Noguchi⁶ in their work on the cytolysis of normal sera was closely followed. Normal goat serum causes rapid swelling and dissolution of the thyroid cells, and the conditions produced resemble those described in their work. The idea occurred that this swelling might be due to a hypotonicity of the serum, and by experiment it was found that the addition of 10 per cent. of a $\frac{n}{1}$ NaCl solution prevented the swelling of the thyroid cells and left them comparatively normal even after several hours. A further study, using the immune and normal serum previously modified in the above manner, showed that shortly after the mixture of serum and the thyroid emulsion an agglutination of the cells occurred with the normal as well as the immune serum, but decidedly more marked with the latter. In one-half to two hours the immune serum caused dissolution of many of the thyroid cells and left many free nuclei, whereas the normal serum only caused some clearing of the granules of the cells. Time did not suffice to carry out as large a number of experiments, modified and refined in various ways, as might be desired.

Specific precipitins: Experiments to test for specific precipitins were conducted with filtered and centrifugalized emulsions of thyroid tissue. Both the normal and immune goat serum caused slight precipitates if much serum was used, but with high dilutions (1:1,000) the immune serum caused a distinct cloudiness, while the normal produced only the faintest reaction or none at all.

Filtered solutions of colloid matter, in salt solution (0.85 per

⁶ *Jour. of Med. Research*, 1902, 9, 251-269.

cent.) or in magnesium sulphate solution (5 per cent.), when treated with the sera likewise gave distinct precipitates, especially marked in the case of the colloid matter immune serum, even in high dilutions (1:1,000). It is only by the use of high dilutions that anything like specific precipitation of filtered thyroid emulsion or of colloid matter is secured by the immune goat serum. To what extent the precipitate formed in such mixtures is dependent upon the presence in them of dog's blood-serum must be left undetermined for the present.

Hemolytic experiments: The serum of the immunized goats is strongly agglutinating and hemolytic for dog's corpuscles, 0.1 c.c. causing complete hemolysis of 0.1 c.c. of dog's blood. The question naturally arose whether this hemolytic property was due to the injection of dog's blood along with the thyroid emulsion. To determine this, the thyroids were first thoroughly washed with salt solution, as previously described, and another goat immunized; but it yielded a serum just as hemolytic as before. Again, the goat immunized with colloid matter likewise gave a hemolytic serum, though not quite as active. In the preparation of the colloid matter the thyroids were not previously freed from blood, and hence it remained possible that the blood might have been responsible for the hemolizing power of the goat serum. To test this a goat was immunized with extracts of dog's blood prepared exactly as the colloid matter. Increasing amounts of dog's blood were used—maximum 50 c.c.—and three injections of the extract given. The serum of the goat, however, did not gain in hemolytic power.

In their hemolytic action on dog's corpuscles the sera of the immunized goats present certain peculiarities that require further study before sufficiently well understood to merit full presentation. It is hoped that this may be accomplished soon. In the meantime it is interesting to find that immunization of goats with bloodless suspension of thyroid cells and with "colloid matter" of dogs causes a marked increase in the hemolytic power of the serum on dog's corpuscles.

That epithelial cells can cause the production of hemolytic substances has been shown by others. Most striking is the

experiment of v. Dungern,⁷ who, by injecting cow's milk, produced in the animal's serum substances not only cytolytic for epithelial cells, but also hemolytic. And the experiments of Pearce,⁸ with heteronephrotoxic and hepatotoxic serum, produced by the injection of washed, bloodless material, show these sera to have been hemolytic. Hence it seems that a strict cellular specificity cannot be claimed for cytotoxins. To what extent the changes observed in the other organs of my dogs were caused by the direct action of thyrotoxin or by the products of the hemolysis set up by the immune serum is another problem that demands further investigation.

SUMMARY AND CONCLUSIONS.

The chief results of the work presented may be summarized as follows:

The serum of goats injected with suspensions of the thyroid gland or with the thyroid colloid matter of dogs acquires many new and striking properties. Injected into dogs, it causes marked symptoms, prominent among which are depression, convulsions, vomiting, rapid breathing, hemoglobinuria, and early death in some cases, and in other animals that lived longer there were present also some fever, lachrymation, loss of weight, and progressive weakness. It cannot be claimed that there has been reproduced the exact picture presented by thyrosectomized dogs.

These clinical manifestations are associated with removal of colloid matter from the acini of the thyroid gland, desquamation and disintegration of the epithelial cells of the acini, followed in time by restitutive processes and the growth of papillary proliferations. The parathyroid bodies and the hypophysis show no changes. The liver, spleen, and kidneys present marked degenerative and pigmentary changes, which in large measure may be the result of the hemolytic properties of the serum injected, although it is possible that thyrotoxic serum contains cytotoxins for the cells of the liver and kidneys also.

In vitro, the thyrotoxic goat serum is more destructive and agglutinating for thyroid cells of the dog than normal goat serum.

⁷ *Munch. Med. Wchnschr.*, 1899, 46, No. 13, 405.

⁸ *Univ. of Penna. Med. Bull.*, 1903, 16, 217-235.

PLATE IV.

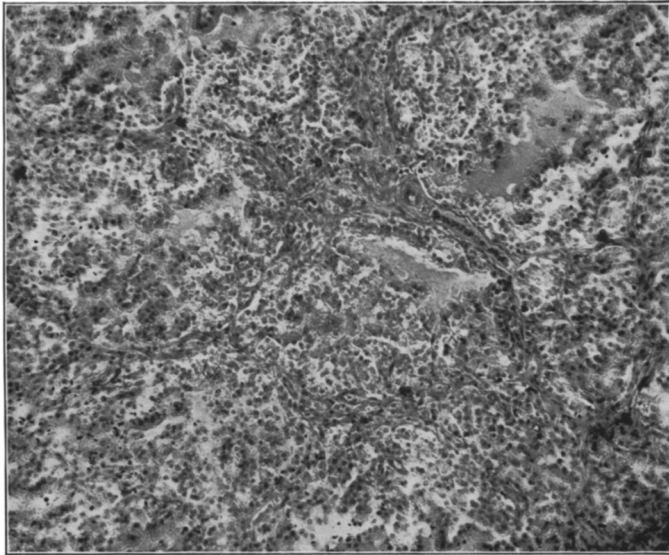


FIG. 1.

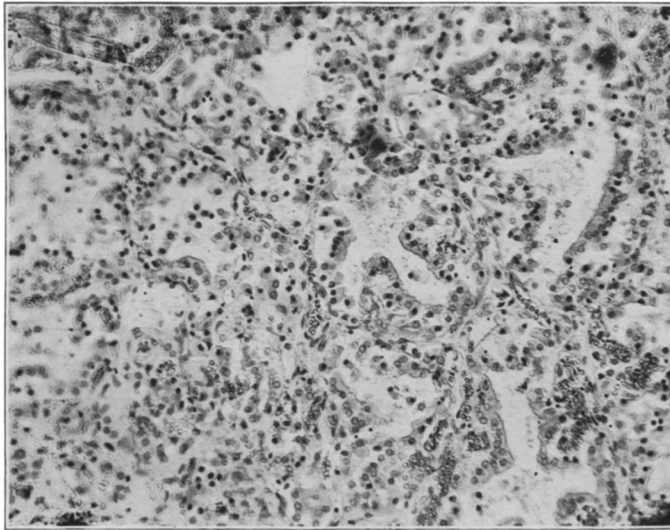


FIG. 2.

PLATE V.

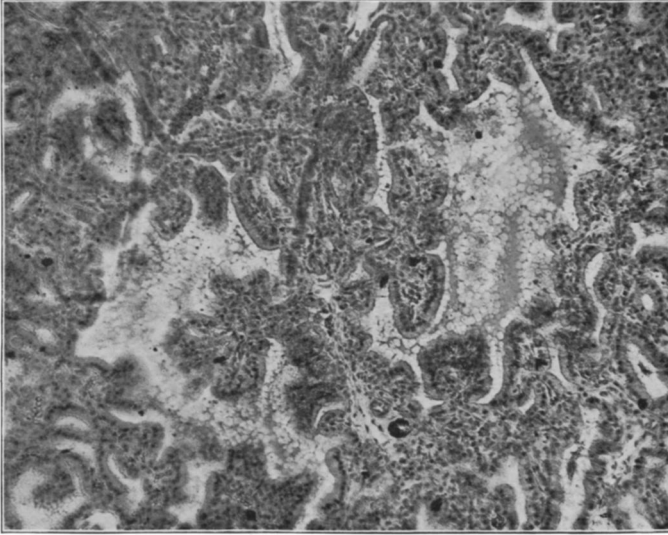


FIG. 3.

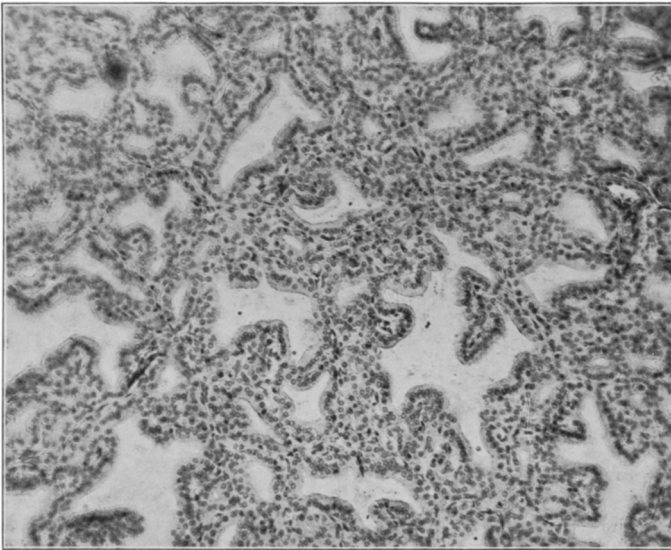


FIG. 4.

The toxic serum is markedly agglutinating and hemolytic for dog's corpuscles, even when obtained from goats injected with bloodless thyroid material and with colloid matter—an observation of great importance as regards the problem of community of receptors in various cells.

If it were possible to remove from the thyrotoxic serum the hemolytic as well as other direct and indirect cytotoxic actions referred to in the foregoing, it would seem to me warranted to expect that still stronger evidence could be obtained of a specific thyrotoxin. It may be, too, that the use of other animals might give less complicated results.

EXPLANATION OF PLATES IV, V.

FIG. 1.—Microphotograph of thyroid of dog No. 1 (see Table I). $\times 125$. Removal of colloid; desquamation and disintegration of epithelial cells.

FIG. 2.—Microphotograph of thyroid of dog No. 19 (see Table I). $\times 175$. Absence of colloid; beginning formation of papillary processes; cellular detritus in some of the acini; congestion.

FIG. 3.—Microphotograph of thyroid of dog No. 24 (see Table I). $\times 125$. Absence of colloid; papillary outgrowths; cellular stroma.

FIG. 4.—Microphotograph of thyroid of dog No. 6 (see Table II). $\times 125$. Same description as in Fig. 3. Blood corpuscles in acini.